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Assays of Plant Extracts

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ICBG PROGRESS REPORT 6/7/2002

Specific Aims

The main objective of these studies was to study the CNS activity of these plant fractions using specific radio ligand binding assays to identify their selectivity profiles as well as to determine their functional activities as either a full or partial agonist or antagonist.

Materials

The following table gives a list of the samples provided to us thus far.

Table 1: ICBG PLANT SAMPLES

Table 1. ICBG I LANT SAWIFLES						
In House Sample#	House Sample# SU-Lab Number		Wt given (mg)			
ICBG 1	SU1904	Whole Plnt	25			
ICBG 2	SU1905	Sd Pulp	25			
ICBG 3	SU1906	Pn polar	25			
	~~~~	i ii potai	23			
ICBG 4	SU1907	Lf/Stem	25			
ICBG 5	SU1908	Lf/stem	25			
10000	501700	Listem	23			
ICBG 6	SU1909	Stbk	25			
TODO	301707	Stok	23			
ICBG 7	SU1910	Stbk	25			
TODG 7	501910	Siuk	25			
ICBG 8	SU1911	C41.1.	0.5			
ICDG 8	301911	Stbk	25			
ICBG 9	SU1912	W71- D1	0.5			
<b>}</b> }		Whole Plnt	25			
ICBG 10	SU1913	Whole Plnt	25			
1000.11	0771011					
ICBG 11	SU1914	Ft pulp	25			

## Methods

*Preparation of Extracts*: Stocks of the solid extracts were prepared by weighing 4 mg of sample and diluting to a total volume of 5 ml with 50% ethanol solvent for a final concentration of 0.8 mg/ml.

Opioid Recetor(kappa-1) Ligand Binding^a: Two-point binding screens using [ 3 H]U69593 were performed as per metheds within the grant and previously (Izenwasser et al,1999). Briefly, mokey insular cortex was homogenized in ice-cold buffer (1:10 w/w, 50 mM Tris, 176  $\mu$ M MnCl₂, 0.1% BSA pH 7.4), centrifuged at 32,000 x g for 10 minutes, and the supernatant was discarded. The pellet was resuspended (1:10, w/w) with buffer, and 100  $\mu$ l was added to each tube for a final concentration of 10.0 mg/ml (original tissue wet weight), in a final assay tube volume of 1.0 ml. Screens were performed by incubating 2 concentrations of extract (0.8 and 80

 $\mu$ g/tube) in the presence 1.0 nM of radioligand for 1 hr at 25°C, to determine their inhibitory potency (% inhibition). Nonspecific binding was determined by binding in the presence of 10  $\mu$ M naloxone. Incubations were terminated by vacuum filtration through glass fiber filters (Whatman 934-AH) presoaked in 1% polyethylenimine (PEI) followed by washing with ice-cold buffer (2 x 5 ml).

Norepinephrine Transporter(NET) Ligand Binding^b: Two-point binding screens using [³H]Nisoxetine were performed as per metheds within the grant and previously (Tejani-Butt et al,1990). Briefly, mokey hypothulamus was homogenized in ice-cold buffer (1:10 w/w, 50 mM Tris, 300 mM NaCl, 5 mM KCl pH 7.4), centrifuged at 32,000 x g for 10 minutes, and the supernatant was discarded. The pellet was resuspended (1:10, w/w) with buffer, and 100 μl was added to each tube for a final concentration of 10.0 mg/ml (original tissue wet weight), in a final assay tube volume of 1.0 ml. Screens were performed by incubating 2 concentrations of extract (0.8 and 80 μg/tube) in the presence 3.0 nM of radioligand for 4 hr at 4°C, to determine their inhibitory potency (% inhibition). Nonspecific binding was determined by binding in the presence of 10 μM Mazindol. Incubations were terminated by adding 4ml of ice-cold buffer and vacuum filtration through glass fiber filters (Whatman 934-AH) presoaked in 0.5% polyethylenimine (PEI) followed by washing with ice-cold buffer (2 x 4 ml).

Data Analysis: Data are analyzed using the non-linear regression algorithms found in EBDA/LIGANDTM and GraphPad PrismTM computer software programs.

Results.

Table 2: Plant Extract Activity (% inhibition) on DAT, SERT, Mμ, Kappa-1 & NET

	DAT	25 420)	SERT		Мμ	Мμ		Kappa-1		Карра-1	
1	$(f^3H)WIN$		([ ¹²⁵ I]RTI-55)			([³H]DAMGO)		([³H]U69593)		([ ³ H]U69593)	
In House	@ 0.8	@ 80	@ 0.8	@ 80	@ 0.8	@ 80	@ 0.8	@ 80	IC50	nΗ	
Sample#	μg/tube	μg/tube	μg/tube	μg/tube	μg/tube	μg/tube	μg/tube	μg/tube	Ug/ml		
ICBG 1		58.7		42.0	1.8	60.4		84.0	37.4	1.21	
ICBG 2		46.3		90.1	16.7	60.2		91.1	15.9	0.93	
ICBG 3		18.8		46.7	31.8	39.4		78.8	51.6	1.32	
ICBG 4		77. <b>7</b>			33.0	41.0		82.1	72.6	1.25	
ICBG 5	_	22.9				33,3			N/A	1.23	
ICBG 6		27.2		20.5		33.4		51.6	68.6	1.49	
ICBG 7						29.6		9.9	N/A	1.42	
ICBG 8		50.6		31.0	10.3	29.2		64.0	44.9	1.04	
ICBG 9		82.0				32.8		64.0	81.8	1.13	
ICBG 10		74.9	3.9	76.1	28.6	32.9		74.5	47.8	1.11	
ICBG 11	<b></b>			87.2	32,4	47.1		41.1	N/A	1,11	

-- denotes no inhibitory activity

As can be seen in Table 2, a number of extracts inhibited greater than 50% of the binding at DAT, SERT and  $M\mu$ . Therefore, full characterization of these compounds will be conducted.

Future Aims

Future studies will be to further characterize those extracts found to be active with full competition curves. In addition we plan to expand the screens with other ligands specific to other neuroreceptors and neurotransporters, and adding new compounds as made available.

### References

^aSari Izenwasser, Julie K. Staley, Stephanie Cohn, and Deborah C. Mash. Characterization of Kappa-Opioid Receptor Binding in Human Insular Cortex. *Life Sciences* 1999; 65(9):857-862.

^bShanaz M, Tejani-Butt, David J, Brunswick and Alan Frazer. [³H]Nisoxetine: a new radioligand for norepinephrine uptake sites in brain. *European Journal of Pharmacology 1990*; **191**:239-243.